
A vignette of discovery

Nancy Flournoy

Department of Statistics

University of Missouri, Columbia, MO

This story illustrates the power of statistics as a learning tool. Through an interplay of exploration and carefully designed experiments, each with their specific findings, an important discovery is made. Set in the 1970s and 80s, procedures implemented with the best intentions were found to be deadly. Before these studies, only hepatitis was known to be transmitted through contaminated blood products. We discovered that the cytomegalovirus could be transferred through contaminated blood products and developed novel blood screening techniques to detect this virus just before it become well known for its lethality among persons with AIDS. We conclude with some comments regarding the design of experiments in clinical trials.

31.1 Introduction

Today blood banks have institutionalized sophisticated procedures for protecting the purity of blood products. The need for viral screening procedures are now taken for granted, but 40 years ago transmission of viral infections through the blood was understood only for hepatitis. Here I review the genesis of a hypothesis that highly lethal cytomegalovirus (CMV) pneumonia could result from contaminated blood products and the experiments that were conducted to test this hypothesis. The question of cytomegalovirus infection resulting from contaminated blood products arose in the early days of bone marrow transplantation. So I begin by describing this environment and how the question came to be asked.

E. Donnell Thomas began to transplant bone marrow into patients from donors who were not their identical twins in 1969. By 1975, his Seattle transplant team had transplanted 100 patients with acute leukemia (Thomas et al., 1977). Bone marrow transplantation is now a common treatment for childhood leukemia with a good success rate for young people with a well-matched donor.

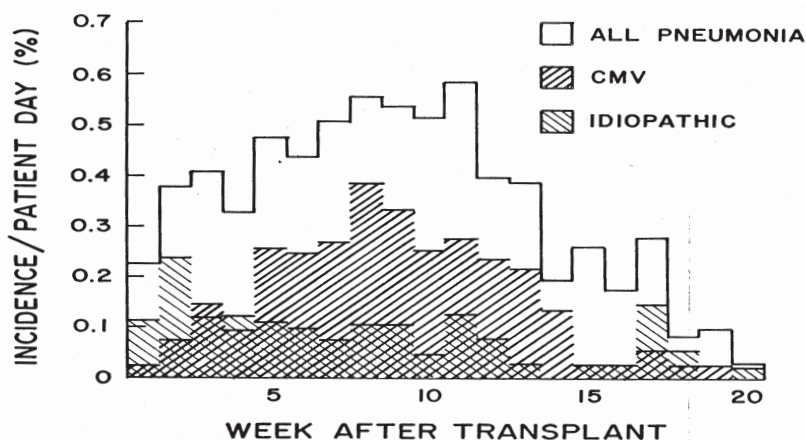
Early attempts to transplant organs, such as kidneys, livers and hearts, were not very successful until it was determined that matching patient and donor at a few key genetic loci would substantially reduce the risk of rejection. Drugs to suppress the natural tendency of the patient's immune system to attack a foreign object further reduced the risk of rejection. In bone marrow transplantation, a good genetic match was also needed to prevent rejection. However, because a new and foreign immune system was being transplanted with the bone marrow, drugs were used not only to reduce the risk of rejection, but to keep the transplanted marrow from deciding that the whole patient was a foreign object and mounting an auto-immune-like attack called graft versus host disease (GVHD). Furthermore, in order both to destroy diseases of the blood and to prevent rejection of the new bone marrow, high doses of irradiation and drugs were given prior to the transplant. Eradicating as completely as possible all the patient's bone marrow destroys the bulk of the patient's existing immune system.

Since typically two to three weeks are required before the transplanted bone marrow's production of blood cells resumes, the Seattle team tried to anticipate problems that could result from a patient having an extended period of severely compromised blood production, and hence extremely poor immune function. It was well known that granulocytes (the white blood cells) fight infection. In order to protect the patient from bacterial infection, an elaborate and expensive system was devised to assure that the patient would be supported with plenty of donated granulocytes. When the transplant team moved into the newly built Fred Hutchinson Cancer Research Center in 1975, a large portion of one floor was dedicated to this task. On rows of beds, the bone marrow donors lay for hours each day with needles in both arms. Blood was taken from one arm, and passed through a machine that filtered off the granulocytes and returned the rest of the blood to the donor through the other arm.

Typically, the same person donated both the bone marrow and the granulocytes, with the hope that the genetic match would prevent the patient from becoming allergic to the granulocyte transfusions. The bone marrow donor was expected to stay in town for at least six weeks and lie quietly with needles in both arms every day so that the transplant patient could fight off threats of infection. Being a marrow donor required a huge time commitment.

31.2 CMV infection and clinical pneumonia

Early in the development of the bone marrow transplant procedure, it was clear that patients with sibling donors who were not identical twins were at high risk of death caused by CMV pneumonia (Neiman et al., 1977). Figure 31.1 — displaying ten years of data from Meyers et al. (1982) — shows

**FIGURE 31.1**

Incidence of CMV and all nonbacterial pneumonias expressed as percentage per patient-day for each week after allogeneic marrow transplant.

the incidence of pneumonia as a function of time following transplantation from genetically matched siblings who were not identical twins. The incidence distribution, expressed as the percentage per patient-day each week, is slightly skewed toward the time of transplant with a mode at about seven weeks. Of 525 patients, 215 (38%) had nonbacterial pneumonia, with CMV isolated in 40% of the cases and other viruses identified in 29% of the cases.

Eighty-four percent of the 215 pneumonia cases were fatal. In contrast, CMV pneumonia was not a cause of death among those patients whose bone marrow donors were identical twins (Appelbaum et al., 1982). At the time, we erroneously speculated that this difference was due to the fact that patients without identical twin donors received more drugs to suppress the immune system than did patients with identical twin donors with no risk of graft rejection. However, the identical twins did not receive their transplant care at the Fred Hutchinson Cancer Research Center, but at University of Washington Hospital, where there was no provision for providing prophylactic granulocytes. We failed to recognize that the twins' reduced drug therapy was confounded with their not getting prophylactic granulocyte transfusions.

CMV is a common virus in the environment and by the time people reach adulthood, it can be found in about half the population. CMV does not cause problems in healthy individuals, but because it was diagnosed most frequently in pneumonia cases and these were the most fatal, the push was on to characterize the course of the illness, identify prognostic factors and find therapies. In order to standardize diagnostic procedures so that the course of the risk period could be established and to identify cases of viral infection early so

that intervention trials might be feasible, we instituted a standard schedule of testing blood and urine samples for the presence of CMV virus and measuring anti-CMV antibody titers. CMV infection was defined to be present if the CMV virus was isolated in the routine blood and urine tests, or if it was found in the course of diagnosing pneumonia, or if antibody titers rose four fold (seroconverted). Between October 1977 and August 1979, surveillance testing of blood and urine samples and antibody to CMV was measured in 158 patients and their donors prior to transplantation and periodically following transplant. The incidence of CMV infection in urine samples was approximately the same, regardless of the presence or absence of antibody to CMV before transplant, in either the donor or the recipient (Meyers et al., 1980). However, antibody titers increased (as measured by a summary statistic, the mean response stimulation index) after 41–60 days following transplant among patients who contracted CMV pneumonia (Figure 31.2). Note how the mode of the simulation index among patients with CMV pneumonia coincides with the time of peak incidence shown in Figure 31.1. Among patients whose CMV titers were positive pretransplant (seropositive), average titers remained high (see Figure 31.3). But among patients whose pretransplant titers were negative (seronegative), the stimulation index remained low until about 60 days after transplant and then began to rise without regard to the marrow donor’s pretransplant titer. So although we dismissed the idea that CMV infection was being transferred through the donor’s bone marrow, this study suggested prognostic factors that might be manipulated in an intervention.

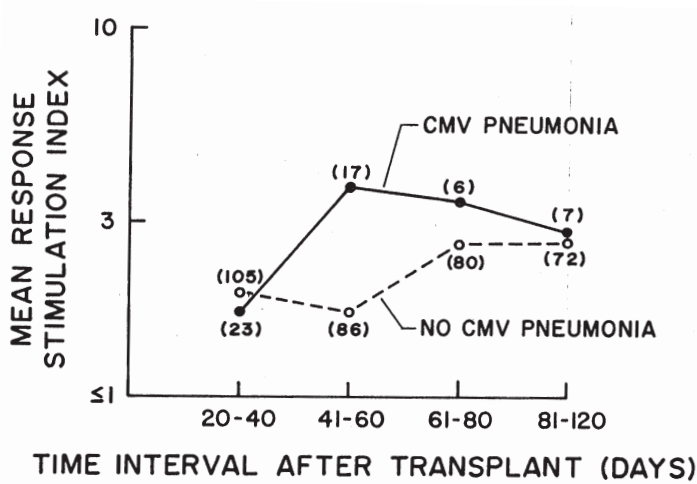
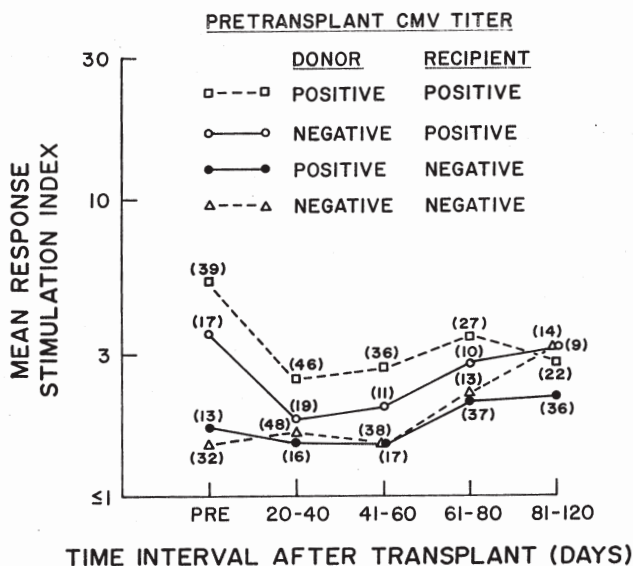


FIGURE 31.2
Mean response of lymphocytes to cytomegalovirus antigen. Numbers in parentheses represent the sample size in each group.

**FIGURE 31.3**

Mean response of lymphocytes to cytomegalovirus. Numbers in parentheses represent the sample size in each group.

Having designed and implemented a multidisciplinary research information system (Flournoy and Hearne, 1990) before the advent of commercial systems, we began mining the data for prognostic factors for CMV infection among those patients transplanted between 1979 and 1982 who had at least four surveillance cultures (Meyers et al., 1986). The surveillance data showed that just over half (51.5%) of the 545 recipients of marrow transplants without an identical twin donor became infected with CMV. CMV was cultured from 280 (51.4%) of the patients; 168 (30.8%) had at least a four-fold rise in titers (seroconverted). Much attention in this study focused on the relationship between the surveillance test results and the subsequent development of pneumonia. Also, the relationship between surveillance results, pneumonia and the complication of the transplant procedure GVHD were investigated. An association between GVHD and CMV clearly existed, suggesting that fatalities due to CMV would be reduced by eliminating GVHD. This was a false lead down another blind alley.

Among patients who had CMV in their blood prior to transplant, 69% subsequently became infected (i.e., they either seroconverted and/or began to excrete CMV in their urine). Among patients without CMV in their blood prior to transplant, 57% of those whose donors did and 28% of those whose donors did not have CMV in their blood subsequently became infected. These

observations suggested that patients having CMV in their blood prior to transplant were at high risk of infections; and that in patients without CMV in their blood prior to transplant, the donor might be passing the infection to the patient, either through the marrow transplant itself or through the blood transfusions given after transplant. This was our first clue that the granulocyte transfusions might be transmitting CMV; but it was impossible to fathom that our large effort dedicated to providing prophylactic granulocyte transfusions was so harmful. We believed that a randomized clinical trial would confirm that there was some unknown confounding variable that would explain away this association.

A proportional hazards regression analysis was performed separately for patients with and without CMV in their blood prior to transplant. Among seropositive patients, all the significant covariates were demographic variables, disease characteristics or treatment complications for which no known control was possible. Thus the models did not suggest possible interventions. However, among seronegative patients, the relative rate of CMV infection was 2.3 times greater ($p = .0006$) if the granulocyte transfusions were also found to be positive for CMV. This was the second clue.

31.3 Interventions

To run a clinical trial of subjects receiving and not receiving prophylactic granulocytes required developing a higher throughput procedure for identifying CMV in blood products. While exploratory discussions began with the King County Blood Bank about how to develop the needed screening procedures, we began an alternative clinical trial that did not require novel blood analytic methods be developed.

In light of the data mining results, we focused on the patients whose pre-transplant titers were negative. Thinking that prophylactic anti-CMV immune globulin might prevent CMV infection from developing, eligible patients were randomized to receive globulin or nothing, with stratifications for the use of prophylactic granulocyte transfusions and the donor's titer to CMV. At the onset of the CMV immune globulin study, we took the association between CMV infection and granulocyte transfusions seriously enough to stratify for it, but not so seriously as to study it directly. To be eligible for this study (Meyers et al., 1983), a patient had to be seronegative for antibody to CMV prior to transplant and to not excrete the virus into the urine for the first two weeks after transplantation. Patients excreting virus during this period were presumed to have been infected with CMV before transplantation and were excluded from final analysis.

Figure 31.4 compares Kaplan–Meier estimates of the probability of CMV infection as a function of week after transplant for globulin recipients and

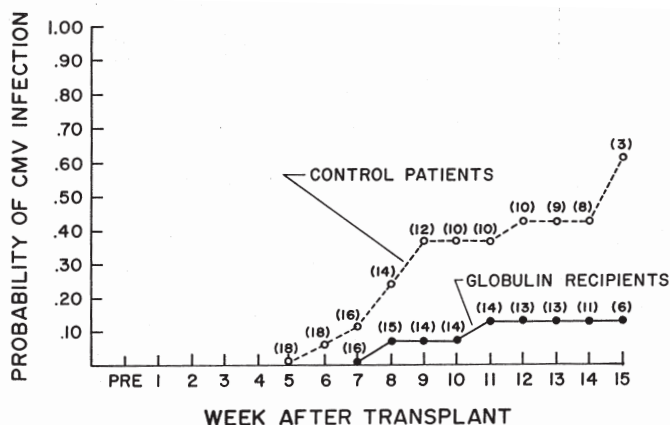


FIGURE 31.4

Kaplan-Meier probabilities of acquiring cytomegalovirus infection. The numbers in parentheses indicate the sample size of patients still at risk of infection at the beginning of each interval. The risk is different for globulin recipients and controls at $p = .03$ by the Mantel-Cox test.

control patients. The overall difference in infection rates between globulin recipients and controls was not significant. CMV infection rates by strata are shown in Table 31.1. Seeking any lead to act upon, the difference observed among patients receiving no granulocytes provided hope that globulin might be effective in a larger study of some subset of subjects. The difference in rates depending upon whether or not the granulocyte donor was seronegative or seropositive finally led us to question seriously the role of granulocyte transfusions in CMV infection.

We were thunderstruck by the possibility that we were transmitting CMV through the blood. The impact should this observation be confirmed in a controlled randomized study is described by Meyers et al. (1983):

“Screening blood products for antibody to cytomegalovirus, or more appropriately for virus or viral antigens (techniques that are not yet available), increases the burden on blood-banking facilities, decreases the pool of blood donors, and, most importantly, decreases the rapid availability of fresh blood products such as platelets. The use of an immune globulin is therefore an attractive practical alternative among patients who need large amounts of fresh blood products such as platelets and for whom screening of blood products is less practical.”

The New England Journal of Medicine rejected our paper (Meyers et al., 1983) because our observations concerning granulocyte transfusions were external to the hypotheses postulated in the initial experimental design. But these

TABLE 31.1
Incidence of CMV infection.

Patient Treatment	Globulin		No Globulin	
Granulocytes from seropositive donors	7/8	(88.5%)	6/7	(85.7%)
Granulocytes from seronegative donors	1/5	(20.0%)	0/6	(00.0%)
No granulocytes	2/17	(11.8%)	8/19	(42.1%)

observations led to new hypotheses and the next randomized study. While it is extremely important to distinguish between observations obtained by controlled randomized intervention and those obtained otherwise, hypothesis generation is an essential task.

We spent a year working with King County Blood Bank to develop screening procedures, set up laboratory equipment and train technicians in order to conduct a randomized clinical trial. Although we restricted the study to patients who were seronegative for CMV in two consecutive tests and who had not received any unscreened blood recently, more patients were available for study than the blood bank could handle. Therefore, we studied the prophylactic capability of immune globulin at the same time in a randomized 2×2 factorial design. CMV immune globulin had no effect [data not shown] on the rate of CMV infection (Bowden et al., 1986).

The effect of only giving CMV seronegative blood transfusions, controlling for the marrow donor’s CMV status, is summarized in Table 31.2.

Among patients whose marrow donors were seronegative, those randomized to receive seronegative granulocyte transfusions had a 4.5% infection rate, whereas those randomized to receive unscreened transfusions had a 32% infection rate. What is more, the one patient with a seronegative donor who was assigned to receive seronegative blood products and subsequently became infected with CMV actually mistakenly received several seropositive transfusions.

TABLE 31.2
Incidence of CMV infection among 85 patients studied for at least 62 days after transplantation.

Marrow Donor’s CMV Status	Randomized to Granulocytes	
	Seronegative	Unscreened
Seronegative	1/22 (04.5%)	8/25 (32.0%)
Seropositive	3/12 (25.0%)	5/16 (31.3%)

As the study proceeded, blood bank personnel became increasingly agitated as they considered the ramifications of a significant finding. Blood banks all over the country would have to set up screening programs; the cost would be enormous, they warned. The study results went out to blood banks, however, and viral screening procedures were put into place. The timing was fortuitous because the AIDS crisis was building. Today the idea that viral infections can be transmitted through the blood is taken for granted.

31.4 Conclusions

Our experience suggests three key steps to successful experimental designs. First, ask an important, well defined question. Too often this step receives insufficient attention and resources, and it may be the most difficult. Determining a well structured and important question can involve considerable data collection, exploratory analysis and data mining. Data mining without data collection targeted to a specific question may yield valuable findings, but many important questions now go begging in the push to devote time and resources to analyzing existing databases. (When medical fellows began rotating through the Fred Hutchinson Cancer Center wards, they had to conceive of a study, execute it and draft a paper. The questions they raised were inventive and some resulted in life-saving findings. When I left in 1986, having created a shared interdisciplinary research information system, the fellows typically looked at what data was available and studied a question that could be answered with the available data. I found the relative lack of imagination in the questions being asked extremely distressing, and feel responsible for enabling it.)

The problem associated with moving too quickly to confirmatory studies has fairly recently been acknowledged by the pharmaceutical industry. But this acknowledgement seems slow to translate into substantially increased resources being devoted to early phase studies.

The second key step is to develop interventions that focus sharply on the question at hand and to randomize subjects to the interventions and to the standard of care or a placebo. This step is operationally well developed. The third step is to replicate the experiment and encourage others to do likewise.

While this series of studies resulted in important discoveries, other series of studies were not so fruitful. In particular, two-arm randomized studies of chemotherapy and radiation schedules were largely uninformative. These variables are virtually continuous, whereas the important variables in the studies of CMV were mostly inherently discrete. With such multidimensional continuous random variables in an environment in which ethics preclude utilizing a large sample space covering unknown territory, our unbridled enthusiasm for the two-arm clinical trial as a learning tool was misplaced. Coming to appre-

ciate the necessity of exploratory analysis in such settings led to my current work in adaptive designs and their analysis.

References

- Appelbaum, F., Meyers, J., Fefer, A., Flournoy, N., Cheever, N., Greenberg, M., Greenberg, P., Hackman, R., and Thomas, E. (1982). Nonbacterial nonfungal pneumonia following marrow transplantation in 100 identical twins. *Transplantation*, 33:265–268.
- Bowden, R., Sayers, M., Flournoy, N., Newton, B., Banaji, M., Thomas, E., and Meyers, J. (1986). Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplant. *New England Journal of Medicine*, 314:1006–1010.
- Flournoy, N. and Hearne, L. (1990). *Quality Control for a Shared Multidisciplinary Database*. Marcel Dekker, New York, pp. 43–56.
- Meyers, J.D., Flournoy, N., and Thomas, E.D. (1980). Cytomegalovirus infection and specific cell-mediated immunity after marrow transplant. *The Journal of Infectious Diseases*, 142:816–824.
- Meyers, J.D., Flournoy, N., and Thomas, E.D. (1982). Nonbacterial pneumonia after allogeneic marrow transplantation. *Reviews of Infectious Diseases*, 4:1119–1132.
- Meyers, J.D., Flournoy, N., and Thomas, E.D. (1986). Risk factors for cytomegalovirus infection after human marrow transplant. *The Journal of Infectious Diseases*, 153:478–488.
- Meyers, J.D., Leszczynski, J., Zaia, J.A., Flournoy, N., Newton, B., Snyderman, D.R., Wright, G.G., Levin, M.L., and Thomas, E.D. (1983). Prevention of cytomegalovirus infection by cytomegalovirus immune globulin after marrow transplantation. *Annals of Internal Medicine*, 98:442–446.
- Neiman, P.E., Reeves, W., Ray, G., Flournoy, N., Lerner, K.G., Sale, G.E., and Thomas, E.D. (1977). A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. *The Journal of Infectious Diseases*, 136:754–767.
- Thomas, E.D., Buckner, C.D., Banaji, M., Clift, R.A., Fefer, A., Flournoy, N., Goodell, B.W., Hickman, R.O., Lerner, K.G., Neiman, P.E., Sale, G.E., Sanders, J.E., Singer, J., Stevens, M., Storb, R., and Weiden, P.L. (1977). One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood*, 49:511–533.